

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 3 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

PLEASE AMEND THIS APPLICATION AS FOLLOWS:

In The Title:

Change the title of the invention to:

-- *In vitro* Process for Producing Multiple Nucleic Acid Copies -- .

In The Claims:

Please cancel claim 1.

Please add new claims 91-142 as follows:

Claim 1 (Canceled Herein)

Claims 2-90 (Previously Canceled in Continuation Request)

91. (NEW) An *in vitro* process for producing more than one copy of nucleic acids of interest, said process comprising the steps of:

- (a) providing a nucleic acid sample containing said nucleic acids of interest;
- (b) contacting said sample with a mixture comprising:
 - (i) nucleic acid precursors;
 - (ii) one or more specific polynucleotide primers comprising at least one ribonucleic acid segment, each of which primer comprises a

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 4 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

sequence complementary to a distinct sequence of said nucleic acids of interest;

(iii) an effective amount of a nucleic acid producing catalyst; and

(iv) RNase H; and

(c) carrying out nucleic acid synthesis, thereby generating multiple copies of said nucleic acids of interest.

92. (NEW) The process of claim 91, wherein said primers (ii) comprise modified nucleotides, unmodified nucleotides or a combination thereof.

93. (NEW) The process of claim 91, wherein said primers (ii) comprise sequences noncomplementary to said distinct sequence of said nucleic acids of interest.

94. (NEW) The process of claim 93, wherein said primers (ii) comprise from about 1 to about 200 noncomplementary nucleotides or nucleotide analogs.

95. (NEW) The process of claim 91, wherein said primers (ii) further comprise deoxyribonucleotides.

96. (NEW) The process of claim 91, wherein said nucleic acid producing catalyst (iii) comprises DNA polymerase, RNA polymerase, reverse transcriptase or a combination thereof.

97. (NEW) The process of claim 96, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria or a combination thereof.

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 5 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

98. (NEW) The process of claim 97, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

99. (NEW) The process of claim 91, wherein said mixture recited in step (b) comprises nucleic acid precursors, one or more specific labeled polynucleotide primers or a combination of both.

100. (NEW) The process of claim 91, wherein said primers comprise a 3'-hydroxyl group or an isosteric configuration of heteroatoms.

101. (NEW) The process of claim 100, wherein said heteroatoms comprise nitrogen or sulfur.

102. (NEW) An *in vitro* process for producing more than one copy of nucleic acids of interest, said process comprising the steps of:

- (a) providing a nucleic acid sample containing or suspected of containing said nucleic acids of interest;
- (b) contacting said sample with a mixture comprising:
 - (i) nucleic acid precursors;
 - (ii) one or more specific polynucleotide primers comprising at least one ribonucleic acid segment and at least one deoxyribonucleic acid segment, each of which primer comprises a sequence complementary to a distinct sequence of said nucleic acids of interest;
 - (iii) an effective amount of a nucleic acid producing catalyst; and

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 6 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

- (iv) RNase H; and
- (c) allowing nucleic acid synthesis to be carried out, thereby generating multiple copies of said nucleic acids of interest.

103. (NEW) The process of claim 102, wherein said primers comprise modified nucleotides, unmodified nucleotides or a combination of both.

104. (NEW) The process of claim 102, wherein said primers comprise sequences noncomplementary to said distinct sequence of said nucleic acids of interest.

105. (NEW) The process of claim 104, wherein said primers comprise from about 1 to about 200 noncomplementary nucleotides or nucleotide analogs.

106. (NEW) The process of claim 102, wherein said nucleic acid producing catalyst (iii) comprises DNA polymerase, RNA polymerase, reverse transcriptase or a combination thereof.

107. (NEW) The process of claim 106, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria, or a combination thereof.

108. (NEW) The process of claim 107, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 7 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

109. (NEW) The process of claim 102, wherein said mixture recited in step (b) comprises nucleic acid precursors, one or more specific labeled polynucleotide primers, or a combination of both.

110. (NEW) The process of claim 102, wherein said primers (ii) contain a 3'-hydroxyl group or an isosteric configuration of heteroatoms.

111. (NEW) The process of claim 110, wherein said heteroatoms comprise nitrogen or sulfur.

112. (NEW) An *in vitro* process for producing more than one copy of nucleic acids of interest, said process comprising the steps of:

- (a) providing a nucleic acid sample containing said nucleic acids of interest;
- (b) contacting said sample with a mixture comprising:
 - (i) nucleic acid precursors;
 - (ii) one or more specific polynucleotide primers comprising at least one ribonucleic acid segment, each of which primer comprises a sequence complementary to a distinct sequence of said nucleic acids of interest;
 - (iii) an effective amount of a nucleic acid producing catalyst; and
 - (iv) RNase H; and
- (c) carrying out nucleic acid synthesis to produce a polynucleotide comprising an RNA/DNA hybrid, thereby generating a substrate for RNase H;

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 8 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

- (d) digesting said substrate with RNase H to remove said ribonucleic acid segment and allow another primer binding event to occur, thereby producing multiple copies of said nucleic acids of interest.

113. (NEW) The process of claim 112, wherein said primers (ii) comprise modified nucleotides, unmodified nucleotides or a combination thereof.

114. (NEW) The process of claim 112, wherein said primers (ii) comprise sequences noncomplementary to said distinct sequence of said nucleic acids of interest.

115. (NEW) The process of claim 114, wherein said primers (ii) comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

116. (NEW) The process of claim 112, wherein said primers (ii) further comprise deoxyribonucleotides,

117. (NEW) The process of claim 112, wherein said nucleic acid producing catalysts (iii) comprise DNA polymerase, RNA polymerase, reverse transcriptase or a combination thereof.

118. (NEW) The process of claim 117, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria or a combination thereof.

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 9 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

119. (NEW) The process of claim 118, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

120. (NEW) The process of claim 112, wherein said mixture recited in step (b) comprises nucleic acid precursors, one or more specific labeled polynucleotide primers, or a combination of both.

121. (NEW) The process of claim 112, wherein said primers (ii) contain a 3'-hydroxyl group or an isosteric configuration of heteroatoms.

122. (NEW) The process of claim 121, wherein said heteroatoms comprise nitrogen or sulfur.

123. (NEW) A process for multiply initiating polynucleotide or oligonucleotide synthesis comprising:

- (a) providing nucleic acids of interest;
- (b) contacting said sample with a mixture comprising:
 - (i) nucleic acid precursors;
 - (ii) one or more specific copolymer primers comprising at least one DNA segment and at least one RNA segment, each of which primer comprises a sequence complementary to a distinct sequence of said nucleic acid of interest;
 - (iii) an effective amount of a nucleic acid producing catalyst; and
 - (iv) RNase H; and

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 10 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

- (c) producing at least one copy of said nucleic acid of interest by using said nucleic acid producing catalyst (iii) and said nucleic acids of interest as templates; and
- d) removing said RNA segment from said template by digesting with RNase H to bind another primer and initiate synthesis, thereby multiply initiating polynucleotide or oligonucleotide synthesis.

124. (NEW) The process of claim 123, wherein said primers comprise modified nucleotides, unmodified nucleotides or a combination thereof.

125. (NEW) The process of claim 123, wherein said primers further comprise sequences that are noncomplementary to said distinct sequence of said nucleic acids of interest.

126. (NEW) The process of claim 125, wherein said primers comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

127. (NEW) The process of claim 123, wherein the nucleic acid producing catalyst (iii) comprises DNA polymerase, RNA polymerase, reverse transcriptase or a combination thereof.

128. (NEW) The process of claim 127, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria or a combination thereof.

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 11 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

129. (NEW) The process of claim 128, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

130. (NEW) The process of claim 123, wherein said mixture recited in step (b) comprises nucleic acid precursors, one or more specific labeled polynucleotide primers or a combination of both.

131. (NEW) The process of claim 123, wherein said primers contain a 3'-hydroxyl group or an isosteric configuration of heteroatoms.

132. (NEW) The process of claim 131, wherein said heteroatoms comprise nitrogen or sulfur.

133. (NEW) An *in vitro* process for producing more than one copy of RNA of interest, said process comprising the steps of:

- (a) providing a nucleic acid sample containing said RNA of interest;
 - (b) contacting said sample containing with a mixture comprising:
 - (i) nucleic acid precursors;
 - (ii) one or more polynucleotide primers wherein said primers comprise (A) at least one ribonucleic acid segment and (B) a sequence complementary to a distinct sequence in said RNA of interest;
 - (iii) an effective amount of a nucleic acid producing catalyst; and
 - (iv) RNase H;
 - (c) producing at least one DNA copy from said RNA of interest;
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Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 12 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

- (d) using said DNA copy as a template to produce a double-stranded copy comprising a second copy complementary to said DNA copy produced in step (c); and
- (e) removing said ribonucleic acid segment of said primers with RNase H from said double-stranded copy produced in step (d) to regenerate a primer binding site, thereby allowing a new priming event to occur and producing more than one copy of said RNA of interest.

134. (NEW) The process of claim 133, wherein said primers (ii) comprise modified nucleotides, unmodified nucleotides or a combination thereof.

135. (NEW) The process of claim 133, wherein said primers (ii) further comprise sequences noncomplementary to said distinct sequence of said RNA of interest.

136. (NEW) The process of claim 135, wherein said primers (ii) further comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

137. (NEW) The process of claim 133, wherein said primers (ii) further comprise deoxyribonucleotides.

138. (NEW) The process of claim 133, wherein said nucleic acid producing catalysts (iii) comprise DNA polymerase, RNA polymerase, reverse transcriptase or a combination thereof.

Engelhardt et al.

Serial No.: Not Yet Assigned

(Continuation of S.N. 10/206,031, filed June 6, 2003)

Filed: Herewith

Page 13 [Preliminary Amendment (Accompanying Continuation Application
Under 37 C.F.R. §1.53(b)) --- November 14, 2003]

139. (NEW) The process of claim 138, wherein said DNA polymerase comprises E. coli DNA polymerase I, Klenow polymerase, polymerases derived from thermophilic bacteria, or a combination thereof.

140. (NEW) The process of claim 139, wherein said polymerases derived from thermophilic bacteria comprise Taq DNA polymerase.

141. (NEW) The process of claim 91, wherein said mixture recited in step (b) comprises nucleic acid precursors, one or more specific labeled polynucleotide primers or a combination of both.

142. (NEW) The process of claim 141, wherein said primers comprise from about 1 to 200 noncomplementary nucleotides or nucleotide analogs.

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